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CACACTTAAAGCTTTCGTCTTTACCTCTTCCCTTCTCTCTCTCTATCTAAAAAGAGTTGGAGA    64
AGAAGATCATCATCAATGGCGACTTCTCTCTTCTTCATGTCAACAGATCAAAACTCCGTCGGAA   128
ACCCAAACGATCTTCTGAGAAACACCCGTCTTGTCGTCAATAGCTCCGGCGAGATCCGGACAGA   192
GACACTGAAGAGTCGTGGTCGGAAACCAGGATCGAAGACAGGTCAGCAAAAACAGAAGAAACCA   256
ACGTTGAGAGGAATGGGTGTAGCAAAGCTCGAGCGTCAGAGAATCGAAGAAGAAAAGAAGCAAC   320
TCGCCGCCGCCACAGTCGGAGACACGTCATCAGTAGCATCGATCTCTAACAACGCTACCCGTTT   384
ACCCGTACCGGTAGACCCGGGTGTTGTGCTACAAGGCTTCCCAAGCTCACTCGGGAGCAACAGG   448
ATCTATTGTGGTGGAGTCGGGTCGGGTCAGGTTATGATCGACCCGGTTATTTCTCCATGGGGTT   512
TTGTTGAGACCTCCTCCACTACTCATGAGCTCTCTTCAATCTCAAATCCTCAAATGTTTAACGC   576
TTCTTCCAATAATCGCTGTGACACTTGCTTCAAGAAGAAACGTTTGGATGGTGATCAGAATAAT   640
GTAGTTCGATCCAACGGTGGTGGATTTTCGAAATACACAATGATTCCTCCTCCGATGAACGGCT   704
ACGATCAGTATCTTCTTCAATCAGATCATCATCAGAGGAGCCAAGGTTTCCTTTATGATCATAG   768
AATCGCTAGAGCAGCTTCAGTTTCTGCTTCTAGTACTACTATTAATCCTTATTTCAACGAGGCA   832
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GATGAAAGTAAGAAACAGAAGAAAAAAAAGAGAGAGCCTTTTTTGGTGTATGCATCTGAGAGCT  1152
GAGTCGAAAGAAAGATTCAGCTTTTGGATTACCCTTTTGGTTGTTTATTATGAGATTCTAACCT  1216
AAACACTCAGACATATATGTTCTGTTCTCTTCCTTAATTGTTGTCATGAAACTTCTCAAAAAAA  1280
AAAAAAAAAAAAAAAAAAAAAA                                            1302
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(57) Abstract

The present invention provides genes and encoded proteins that are involved in meiocyte formation during the growth of plants. The transformation of plants and plant-related hosts with these genes in altered or unaltered form, or the mutation of these genes in endogenous form, renders a plant capable during growth of bearing seedless fruits and/or pollenless flowers. The invention further provides methods of producing transgenic plants which are capable of bearing seedless fruits and/or pollenless flowers.

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CONTROL OF SPOROCYTE OR MEIOCYTE FORMATION IN PLANTS

Field of the Invention

The present invention relates to genes and encoded proteins involved in fertility of plants. More particularly, the present invention relates to the use of genes and encoded proteins involved in meiocyte formation in plants to render plants capable of bearing seedless fruits and/or pollenless flowers.

Background of the Invention

A fundamental part of the life cycle of higher plants is the alternation between a diploid, sporophytic generation and a haploid, gametophytic generation. In flowering plants, the gametophytic generation consists of pollen grains and the embryo sac within the ovary. The transition from the sporophytic phase to the gametophytic phase in higher plants consists of two processes, sporogenesis and gametogenesis. Gametogenesis mainly involves the differentiation of haploid spores into mature gametophytes. See G.N. Drews, et al., *Plant Cell* 10(5) (1988). Sporogenesis is characterized by the differentiation of hypodermal cells in anthers and ovule primordia into archesporial cells that further

develop into microsporocytes (pollen mother cells) and megasporocytes (egg mother cells). See J. Bowman, (1994) *Arabidopsis, An Atlas of Morphology and Development*. The microsporocytes and megasporocytes (collectively known as meiocytes) undergo meiosis to produce spores. The formation of meiocytes thus comprises a very important step in plant reproduction.

In *Arabidopsis*, sporogenesis and gametogenesis (also known as megasporogenesis and megagametogenesis, respectively) have been well described. See Bowman, J., 1994, *Arabidopsis, An Atlas of Morphology and Development*. In sporogenesis, bitegmic and tenuinucellate ovules arise as finger-like structures on the placenta in the ovary (carpel) of the plant. A single hypodermal cell at the top of the ovule primordia becomes more prominent than neighboring cells because of its slightly larger size, denser cytoplasm and more conspicuous nucleus, and differentiates into an archesporial cell in stage 10-11 flowers. The archesporial cell then elongates and polarizes its cellular components longitudinally and differentiates into a sporocyte or megaspore mother cell (MMC). The MMC then undergoes meiosis to form four haploid megaspores (tetrad). Shortly after the archesporial cell becomes visible, in stage 11 flowers, the inner and outer integuments form from epidermal cells at the base of the nucellus. In gametogenesis, the outer integument overgrows the inner integument and both inner and outer integuments envelop the nucellus in which the female gametophyte (embryo sac) develops during stage 13. At mature stage, the inner cell layer

of the inner integument differentiates into a nutritive endothelium (integumentary tapetum).

Although the above is well known, little is known about the molecular and genetic mechanisms that regulate and control sporogenesis, especially meiocyte formation. The identification of genes that regulate and control meiocyte formation could help understand these mechanisms and find ways to manipulate the fertility of plants.

An object of the present invention thus is to provide isolated nucleic acids and encoded proteins involved in meiocyte formation in plants, which can be used to manipulate plant fertility.

Another object of the present invention is to produce plants in which meiocyte formation has been affected during growth to render the plant capable of bearing altered fruits and/or altered flowers, including seedless fruits and pollenless flowers.

Summary of the Invention

The present invention relates to the identification of a new gene *Sporocyteless* (*SPL*) that is involved in meiocyte formation in both male and female organs in plants. The *SPL* gene, its encoded polypeptides and proteins, and their homologues, can be utilized to regulate and control meiocyte formation in plants in order to produce altered plants, including plants that are capable of bearing seedless fruits or pollenless flowers, or fruits and flowers which are substantially seedless and pollenless, respectively.

In accordance with one embodiment of the present

invention, there are provided isolated nucleic acids and their complements that encode proteins involved in the formation of meiocytes in plants. These isolated nucleic acids include DNA, or portions thereof, of the *SPL* gene isolated from *Arabidopsis thaliana* ecotype landsberg *erecta* plant and other plant species. The invention also provides homologues of the *SPL* gene from *Arabidopsis* and other plant species that can hybridize to DNA of the *SPL* gene. These homologues demonstrate *SPL*-type function and can be identified throughout the plant kingdom.

The DNA in accordance with the present invention may exist in various forms, including exogenous DNA that encodes a protein involved in regulating or controlling meiocyte formation in a plant.

The DNA of the present invention also may be exogenous DNA that has been altered by mutation or other means to affect meiocyte formation in a plant. In a preferred embodiment, the present invention provides for the insertion of genetic elements, such as *Ds* sequences (with or without active Ac sequences) into the above-described nucleic acids.

The present invention further provides for alteration or mutation of a plant's endogenous DNA responsible for meiocyte formation, by direct or targeted mutagenesis, or other technique, which also may affect meiocyte formation. A plant containing the mutated gene thus may be capable of bearing seedless fruits and/or pollenless flowers.

In accordance with the present invention, there are also provided polypeptides or proteins involved in

meiocyte formation in plants. These polypeptides or proteins can regulate or control meiocyte formation and include the SPL protein, or portions thereof, of plant origin. SPL proteins from most or all plant species, or homologues of these proteins that demonstrate the same or similar regulatory function (i.e., meiocyte formation) as SPL protein, also are encompassed by this invention. A homologous polypeptide is defined herein as one having an amino acid sequence with at least about 80% or greater homology to the amino acid sequence drawn in Figure 3 [SEQ ID NO:4].

In another respect, this invention relates to antibodies that bind the polypeptides and proteins described herein. Such antibodies may be used to localize sites of regulatory activity in plants. In accordance with another embodiment of the invention, fusion proteins comprising the SPL protein and an additional peptide, such as a protein tag, also can be used to detect sites of SPL protein/protein interaction in plants.

The present invention further provides isolated nucleic acids and their complements useful as hybridization probes for detecting homologous nucleic acids which are involved in meiocyte formation in plants.

The present invention further provides plants and plant-related hosts, including seeds, plant tissue culture, and plant parts, containing DNA which may be altered or unaltered exogenous DNA, or altered endogenous DNA, or portions thereof, which in many ways may be capable of affecting meiocyte formation during plant growth.

In a further embodiment of the present invention, there are provided methods for producing transgenic plants in which meiocyte formation is affected or controlled, and more particularly methods for producing transgenic plants that are capable of bearing seedless fruits and/or pollenless flowers.

The invention further provides the promoter of the *SPL* gene which can be used to drive the expression of the *SPL* gene or a foreign gene in microsporocytes and megasporocytes of plants. The promoter can be used to permit expression of transgene in the reproductive cells of the plant so as to render the plant sterile. The promoter also can be used to express certain genes so as to result in the next generation of seeds from the plant having an altered DNA structure from that of the parent plant.

Brief Description of the Figures and Sequence Listing

Fig. 1A [SEQ ID NO:2] shows a portion of the genomic sequence of the *SPL* gene immediately flanking the Ds sequence (indicated by bold letters). Insertion of the Ds sequence causes a 4 base pair duplication (indicated by underlining) at the insertion site.

Fig. 1B [SEQ ID NO:3] shows the Ds sequence, as shown in Fig. 1A [SEQ ID NO:2].

Fig. 2 [SEQ ID NO:1] shows the cDNA sequence of the *SPL* gene. The codons in bold, atg and taa, indicate the start and stop codons, respectively, of the open reading frame. The underlined sequence, gcta, indicates the insertion site of the Ds sequence.

Fig. 3 [SEQ ID NO:4] shows the amino acid sequence

of the SPL polypeptide, as deduced from the DNA sequence of Fig. 2 [SEQ ID NO:1]. The codons Val Leu (in bold) are located at the insertion site of the Ds sequence.

Sub A1 Fig. 4 [SEQ. ID NOs:5-14] illustrates the alignment of the first 18 amino acids of the MADS domains from several MADS box transcription factors with amino acids 64 to 80 of the SPL protein.

Fig. 5 [SEQ. ID NO:15] shows the DNA sequence of the promoter of the *SPL* gene and the coding region of the gene. The promoter sequence begins 2690 nucleotides upstream of the start codon of the *SPL* gene. The first nucleotide of the start ATG codon is designated as position +1. The start codon ATG and the stop codon TAA are underlined, and two exons are shown in bold.

Detailed Description of the Invention

Sub A2 As stated above, the present invention provides isolated nucleic acid molecules (e.g., DNA or RNA) that encode proteins which are involved in, and may be essential to, the formation of meiocytes in the male and female organs of plants. The nucleic acid molecules described herein are useful for producing *Sporocyteless* (SPL) proteins and SPL-type proteins of plant origin when such nucleic acids are incorporated into any of a variety of protein expression systems known to those skilled in the art. An isolated *SPL* gene in accordance with the present invention is shown in Figure 2 [SEQ ID NO:1]. The sequence of the promoter region of the *SPL* gene, as well as the coding region of the gene is shown in Figure 5 [SEQ. ID

NO:151.

An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

The term "*SPL*" represents the wild type form, while "*spl*" represents the mutated form of a *SPL* gene. The term "SPL" (no italics) represents the wild type form of the protein described herein.

As used herein, a "portion" or "fragment" of the *SPL* gene is defined as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides. This definition includes all sizes in the range of 8-40 nucleotides as well as

greater than 40 nucleotides. Thus, this definition includes nucleic acids of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or nucleic acids having any number of nucleotides within these ranges of values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc., nucleotides), or nucleic acids having more than 500 nucleotides. The present invention includes all novel nucleic acids having at least 8 nucleotides derived from Figures 1A [SEQ ID NO:2] or 2 [SEQ ID NO:1], its complement or functionally equivalent nucleic acid sequences. The present invention does not include nucleic acids which exist in the prior art. That is, the present invention includes all nucleic acids having at least 8 nucleotides derived from Figures 1A [SEQ ID NO:2] or 2 [SEQ ID NO:1] with the proviso that it does not include nucleic acids existing in the prior art.

The *SPL* gene according to an embodiment of the present invention can be derived from a dicotyledon, *Arabidopsis thaliana*. The polypeptide encoded by this gene can regulate or control, and may be necessary for, meiocyte formation in a plant. By mutating the *SPL* gene, a plant becomes unable or less able to produce spores, embryo sac and pollen grain. Therefore, the isolated *SPL* gene of the present invention can be used to generate modified plants, including plants that produce seedless fruits, pollenless flowers and/or have a larger biomass.

The present invention provides isolated nucleic acids or their complements encoding a protein involved in meiocyte formation, wherein said nucleic acids

include: (a) DNA encoding the amino acid sequence set forth in Figure 3 [SEQ ID NO:4], or (b) naturally occurring DNA, or DNA degenerate to the naturally occurring DNA, that hybridizes to the DNA of (a) under moderately stringent conditions, wherein the naturally occurring DNA has at least 70% identity to the DNA of (a), and wherein said naturally occurring DNA encodes protein involved in meiocyte formation.

The present invention further comprises isolated nucleic acids or their complements encoding a protein involved in meiocyte formation in plants, wherein the nucleic acids comprise naturally occurring DNA, or DNA degenerate to the naturally occurring DNA, from plants that hybridize to the DNA of (a) Figure 1A [SEQ ID NO:2], or a portion thereof, or (b) Figure 2 [SEQ ID NO:1], or a portion thereof, under moderately stringent conditions, wherein the naturally occurring DNA has at least about 70% identity to the DNA of (a) or (b), and wherein the naturally occurring DNA encodes such protein.

The present invention further provides isolated nucleic acids or their complements having at least about 70% identity to (a) nucleotides 81 - 1024 of Figure 2 [SEQ ID NO:1], or a portion thereof, or (b) variations of (a) which encode the same amino acid sequence as encoded by (a), but employ different codons for some of the amino acids, and wherein the nucleic acids encode a protein involved in meiocyte formation in plants.

Hybridization refers to the binding of complementary strands of nucleic acid (i.e., sense:antisense strands or probe:target-DNA) to each

other through hydrogen bonds, similar to the bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target DNA can be readily varied by those skilled in the art.

As used herein, the phrase "moderately stringent" hybridization refers to conditions that permit target DNA to bind a complementary nucleic acid that has about 60%, preferably about 70%, more preferably about 75%, even more preferably about 85% homology to the target DNA; with greater than about 90% homology to target DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5×Denhart's solution, 5×SSPE, 0.2% SDS at 42°C, followed by washing in 0.2×SSPE, 0.2% SDS, at 65°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers.

The terms "homology" or "homologue," or to say that a nucleic acid or fragment thereof is "homologous" to another nucleic acid, means that when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 50% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases.

To determine homology between two different nucleic acids, the percent homology may be determined

using the BLASTN program "BLAST 2 sequences." This program is available for public use from the National Center for Biotechnology Information (NCBI) over the Internet (http://www.ncbi.nlm.nih.gov/gorf/bl2.html) (Altschul et al., 1997). The parameters to be used include the combination of the following parameters which yields the highest calculated percent homology (as calculated below with the default parameters shown in parentheses):

Program - blastn
Matrix - 0 BLOSUM62
Reward for a match - 0 or 1 (1)
Penalty for a mismatch - 0, -1, -2 or -3 (-2)
Open gap penalty - 0, 1, 2, 3, 4 or 5 (5)
Extension gap penalty - 0 or 1 (1)
Gap x_dropoff - 0 or 50 (50)
Expect - 10

Along with a variety of other results, the BLASTN program shows a percent identity across the complete strands or across regions of the two nucleic acids being matched. The program shows as part of the results an alignment and identity of the two strands being compared. If the strands are of equal length, the identity will be calculated across the complete length of the nucleic acids. If the strands are of unequal lengths, the length of the shorter nucleic acid is to be used. If the nucleic acids are similar across only a portion of their sequences, the BLASTN program will show an identity across only these similar portions, which are reported individually. For purposes of determining homology herein, the percent homology refers to the shorter of the two sequences

being compared. If any one region is shown in different alignments with differing percent identities, the alignments which yield the greatest homology are to be used.

Alternatively, "homology" exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See Kanehisa, 1984, Nucl. Acids Res. 12:203-13. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions

will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. See, e.g., Wetmur and Davidson, 1968, J. Mol. Biol. 31:349-70.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

The SPL nucleic acid may be that shown in Figure 2 [SEQ ID NO:1] or it may be an allele or a variant or derivative differing from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to the nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in Figure 2 [SEQ ID NO:1], yet encode a polypeptide with the same amino acid sequence as shown in Figure 3 [SEQ ID NO:4]. That is, nucleic acids of the present invention include sequences which are

degenerate as a result of the genetic code. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in Figure 3 [SEQ ID NO:4]. Nucleic acid encoding a polypeptide which is an amino acid sequence variant, derivative or allele of the amino acid sequence shown in Figure 3 [SEQ ID NO:4] is also provided by the present invention.

The *SPL* gene also refers to (a) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in Figure 3 [SEQ ID NO:4] under highly stringent conditions (See Ausubel et al., 1992, Current Protocols in Molecular Biology, (John Wiley and Sons, New York, New York)) and (ii) encodes a gene product functionally equivalent to SPL protein, or (b) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in Figure 3 [SEQ ID NO:4] under less stringent conditions, such as moderately stringent conditions (Ausubel et al., 1992) and (ii) encodes a gene product functionally equivalent to SPL protein. The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

In accordance with a preferred embodiment of the present invention, there is provided an isolated nucleic acid or its complement comprising the same contiguous nucleotide sequence as set forth in Figure 2 [SEQ ID NO:1], or a portion thereof, which encodes a protein involved in meiocyte formation in plants.

Sub A37 There also is provided an isolated nucleic acid sequence or its complement or which hybridizes to said sequence which comprises the contiguous nucleotide sequence as set forth in Figure 2 or a portion thereof which is preceded by a nucleic acid sequence which provides the promoter region of the gene. A nucleotide sequence which provides the promoter region is shown in Figure 5. Specifically, the promoter comprises the sequence located within nucleotide positions -2690 to -1 of the sequence set forth in Figure 5 [SEQ ID NO:15], or functional fragments thereof capable of regulating expression of an operably linked gene.

In one embodiment of this invention, the isolated *SPL* promoter can be operably linked to, and control the expression of, foreign genes.

In accordance with another preferred embodiment of the present invention, there is provided an isolated nucleic acid or its complement comprising the same contiguous nucleotide sequence as set forth in nucleotides 81 - 1024 of Figure 2 [SEQ ID NO:1], or a portion thereof, which encodes a protein involved in meiocyte formation in plants.

In accordance with another embodiment of the present invention, there are provided isolated nucleic acids and their complements encoding polypeptides and proteins that are involved in meiocyte formation in plants. Such involvement may include regulating or controlling meiocyte formation. The polypeptides and proteins encoded by the isolated nucleic acids comprise an amino acid sequence having at least about 80%, more preferably about 90% amino acid identity to the reference amino acid sequence in Figure 3 [SEQ ID

NO:4]; with greater than about 95% amino acid sequence identity being especially preferred. In a preferred embodiment, the invention provides an isolated nucleic acid and its complement comprising a nucleic acid encoding a protein which comprises the same amino acid sequence as set forth in Figure 3 [SEQ ID NO:4].

The SPL polypeptide of the invention thus may be that shown in Figure 3 [SEQ ID NO:4] which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated. The polypeptide may, if produced by expression in a prokaryotic cell or produced synthetically, lack native post-translational processing, such as glycosylation. Alternatively, the present invention is also directed to polypeptides which are sequence variants, alleles or derivatives of the SPL polypeptide. Such polypeptides may have an amino acid sequence which differs from that set forth in Figure 3 [SEQ ID NO:4] by one or more of addition, substitution, deletion or insertion of one or more amino acids.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Preferred substitutions are ones which are conservative, that is, one amino acid is replaced with one of similar shape and charge.

Conservative substitutions are well known in the art and typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and tyrosine, phenylalanine.

Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules or binding sites on proteins interacting with the SPL polypeptide. Since it is the interactive capacity and nature of a protein which defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. In making such changes, the hydrophobic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is generally understood in the art. See Kyte and Doolittle, 1982, *J. Mol. Biol.* 157:105-32. Alternatively, the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The importance of hydrophilicity in conferring interactive biological function of a protein is generally understood in the art (U.S. Patent 4,554,101). The use of the hydrophobic index or hydrophilicity in designing polypeptides is further discussed in U.S. Patent 5,691,198.

In another embodiment of the present invention, there is provided isolated DNA molecules comprising DNA having at least eight consecutive nucleotides of bases 81 - 1024 of Figure 2 [SEQ ID NO:1], or a complement thereof. In a more preferred embodiment of the invention, the isolated DNA molecule has at least 15 consecutive nucleotides of bases 81 - 1024 of Figure 2 [SEQ ID NO:1].

In accordance with another embodiment of the invention, there is provided isolated nucleic acids, or their complements, comprising nucleic acid coding for a mutant SPL polypeptide which blocks, reduces or increases the formation of meiocytes in a plant.

In accordance with another embodiment of the present invention, there is provided a method for the recombinant production of SPL and SPL-type proteins by expressing the above-described nucleic acid sequences in suitable host cells. The proteins can be expressed under the control of the promoter of the *SPL* gene.

In another embodiment of the present invention, there are provided methods of producing transgenic plants which are capable of bearing seedless fruits and/or pollenless flowers, or fruits and flowers which are substantially seedless and pollenless, respectively. These methods include the step of transforming a plant with a suitable expression system comprising the above-described nucleic acid sequences in altered form (e.g., mutated) to block, reduce or increase meiocyte formation in the plant. Persons of ordinary skill in the art can readily determine suitable expression systems. For example, genes under the control of a suitable promoter can be easily

transformed in most crop plants by *Agrobacterium*-mediated and/or biolistic methods. See P. Christou, *Trends in Plant Science* 1:423-431.

Additional embodiments of methods of producing transgenic plants which are capable of bearing seedless fruits and/or pollenless flowers in a plant include the step of transforming a plant with the above-described nucleic acid sequences to block, reduce or increase meiocyte formation by using antisense and related techniques. Sense and antisense technology are routine methods to alter plant development and metabolism. For example, see Jorgensen, R.A. et al. *Plant Mol. Biol.* 31(5):957-73 (1996). The sense and antisense constructs can be introduced readily into plant cells by *Agrobacterium*-mediated and/or biolistic methods. See P. Christou, *Trends in Plant Science* 1:423-431.

In another embodiment, the present invention relates to methods of producing seedless fruits and/or pollenless flowers in a plant comprising the step of expressing in the plant the above-described nucleic acid sequences in altered form to affect meiocyte formation in the plant.

In a preferred embodiment of the present invention, the above-described method comprises the step of transforming a plant with an expression system comprising a nucleic acid or its complement involved in the formation of meiocytes, comprising: (a) nucleic acid encoding a protein according to Figure 3 [SEQ ID NO:4], (b) a nucleic acid as set forth in Figure 2 [SEQ ID NO:1], or a portion thereof, or (c) a nucleic acid as set forth in nucleotides 81 - 1024 of Figure 2 [SEQ

ID NO:1], or a portion thereof, wherein the nucleic acids are mutated to block, reduce or increase the formation of meiocytes in the plants, thereby rendering the plant capable of bearing seedless fruits or pollenless flowers.

In another embodiment of the present invention, there is provided a method of producing a plant capable of bearing seedless fruits or pollenless flowers, comprising the step of mutating endogenous DNA of the plant responsible for the formation of meiocytes, wherein the formation of meiocytes is affected and the plant becomes capable of producing seedless fruits or pollenless flowers, or fruits and flowers which are substantially seedless and pollenless, respectively. In a preferred embodiment of the invention, the endogenous DNA is mutated by direct mutagenesis. See Mazzucato. A., et al., *Development* 125(1):107-114 (1998).

"Transgenic plants" include plants that contain endogenous or exogenous DNA or RNA not occurring naturally in the wild type (native) plant or known variants, or contain additional or inverted copies of naturally-occurring DNA which is introduced as described herein, their progeny, whether produced from seeds, by vegetative propagation, cell, tissue or protoplast culture, or the like. Transgenic plants of the present invention may contain DNA encoding SPL protein or SPL-like proteins involved in meiocyte formation in the plant. For example, when introduced into and/or present in plant cells, the expression of *SPL* DNA or altered versions of *SPL* DNA may produce a plant lacking meiocytes or having more than the normal

number of meiocytes found in untransformed plants of the same variety. For example, the maize *macI* mutant having an excess number of meiocytes causes complete male sterility and partial female sterility. The mechanism by which an excess of meiocytes results in sterility is currently unknown. See Sheridan, W.F., et al., *Genetics* 142:1009-1020 (1966).

The DNA in accordance with the present invention can be exogenous DNA added in a sense or antisense orientation and which encodes a protein involved in, and which may be required for, meiocyte formation in a plant. See Jorgensen, R.A., et al., *Plant Mol. Biol.* 31(5):957-73 (1996). The DNA of the present invention also can be exogenous DNA that has been altered (e.g., by mutation) so that it blocks, reduces or increases meiocyte formation. For example, the insertion of genetic elements, such as *Ds* sequences (with or without active Ac sequences) can affect meiocyte formation, and thus is of particular use in the present invention. The present invention further provides for direct or targeted mutagenesis of a plant's endogenous DNA responsible for meiocyte formation, which also can affect meiocyte formation.

Exogenous and endogenous DNA involved in meiocyte formation which have been mutated by direct mutagenesis differ from the corresponding wild type (naturally-occurring) DNA in that these sequences contain a substitution, deletion or addition of at least one nucleotide and can encode proteins which differ from the corresponding wild type protein by at least one amino acid residue. As used herein, the term

"nucleotide" includes a residue of DNA or RNA.

Exogenous DNA, in altered or unaltered form, can be introduced into the target plant by well-known methods, such as *Agrobacterium*-mediated transformation, microprojectile bombardment, microinjection or electroporation. See, Wilkinson, J.Q., et al., *Nature Biotechnology* 15(5):444-447 (1997).

Plant cells carrying exogenous *SPL* or *SPL*-like DNA, or endogenous *SPL* DNA mutated by direct mutagenesis, can be used to generate transgenic plants in which meiocyte formation is blocked, reduced or increased, and therefore be sources of additional plants, either through seed production or non-seed, asexual reproductive means (i.e., cuttings, tissue culture, and the like).

The present invention also provides plants, plant cells, and plant seed transformed with the above-described nucleic acid sequences. The formation of meiocytes can be affected in such transformed plants, plant cells, and plant seeds during meiocyte formation and during growth of plants.

In accordance with another embodiment of the present invention, there is provided a family of isolated proteins which can regulate or control the formation of meiocytes in male and female organs in plants. Such proteins include proteins that are functionally and structurally related to SPL and so are able to render a plant capable of bearing seedless fruits and/or pollenless flowers by interfering with the function of SPL. Such proteins also include related proteins from other plant species which are

functional and structural equivalents of SPL in those species and perform the same function that SPL performs in Arabidopsis. An exemplary amino acid structure of the proteins of the present invention is set forth in Figure 3 [SEQ ID NO:4]. The proteins of the present invention are involved in the formation of meiocytes in plants and comprise an amino acid sequence having at least about 80%, more preferably about 90% amino acid identity to the reference amino acid sequence in Figure 3 [SEQ ID NO:4]; with greater than about 95% amino acid sequence identity being especially preferred. In a preferred embodiment, the invention provides proteins which comprise or have the same amino acid sequence as set forth in Figure 3 [SEQ ID NO:4].

In accordance with another embodiment of the present invention, there are provided antibodies generated against the above-described proteins. Such antibodies may be employed in various applications, including to localize sites of regulatory activity in plants.

In another embodiment of the present invention, fusion proteins are provided which can comprise any of the above-described amino acids, and in a preferred embodiment, an SPL or SPL-type protein. The fusion proteins in accordance with the present invention also can comprise an additional peptide, such as a protein tag, which may be used to detect sites of SPL protein/protein interaction in plants.

In accordance with yet another embodiment of the invention, the nucleic acid molecules described herein (or fragments thereof) can be labeled with a readily

detectable substituent and used as hybridization probes for assaying for the presence and/or amount of *SPL* or *SPL*-type DNA or RNA in a sample from a given plant species. In a preferred embodiment of the invention, isolated nucleic acid useful as a hybridization probe comprises a nucleic acid having a sequence of nucleotides as set forth in Figures 1A [SEQ ID NO:2] or 2 [SEQ ID NO:1], or a portion thereof. In a more preferred embodiment of the invention, the hybridization probe can be a nucleic acid comprising a nucleic acid having a sequence of nucleotides as set forth in nucleotides 81 - 1024 of Figure 2 [SEQ ID NO:1], or a portion thereof. The nucleic acid molecules described herein, and fragments thereof, also are useful as primers and/or templates in a PCR reaction for amplifying genes encoding *SPL* protein or *SPL*-type proteins described herein.

Sub A47 Another embodiment of the invention provides an isolated promoter of the *SPL* gene. A fragment of DNA extending from 2690 nucleotides upstream of the start codon of the *SPL* gene has been identified as regulating expression of the *SPL* gene. The sequence of this promoter is shown in Figure 5 (SEQ. ID NO: 15) as the sequence from base pair -2690 to -1 in the sequence. The first nucleotide of the start ATG codon is designated as position +1 in the sequence. The sequence from -2690 to -1 is sufficient to give *SPL*-specific expression in megasporocytes and microsporocytes. As used herein, "promoter" includes this sequence, a sequence which hybridizes to this sequence and promotes expression of a coding sequence

operably linked thereto, and functional fragments of this sequence which are capable of promoting or regulating expression of a coding sequence operably linked thereto. The promoter can be operably linked to a coding sequence if it is linked to the ATG start codon of the coding sequence.

The promoter of the *SPL* gene can be used to drive expression of the *SPL* gene or of a foreign gene in microsporocytes and megasporocytes of plants. One utility of the promoter is to permit expression of transgenes specifically in the reproductive cells of the plant. If a transgene, such as a gene encoding a ribonuclease, is expressed under the control of the *SPL* promoter, the plants will be rendered sterile. Alternatively, the *SPL* promoter can be used to express genes encoding transposases or recombinases (proteins that catalyze DNA rearrangements) specifically in reproductive cells (sporocytes), such that the next generation of seeds will have an altered DNA structure from the parent plant. For example, a plant carrying a Cre recombinase under the control of the *SPL* promoter can be used to excise segments of transgenic DNA specifically from the sporocytes. As a result, the parent plant will carry the transgenes, but the progeny will lack the transgene. This result is helpful when it is desired to prevent the spread of transgenes from one generation to the next.

The following studies were conducted in connection with the present invention and are not to be construed as limiting the scope of the present invention.

Mutations in the recessive *spl* gene were

identified during screening of gene trap lines in *Arabidopsis thaliana* ecotype landsberg *erecta*. From a finding that these mutations caused male and female sterility in the plant, it was concluded that the *SPL* gene plays a pivotal role in plant reproduction. The *spl* homozygous plants also exhibited an overall morphology that was similar to the morphology of wild type plants, except for a delay in senescence in the *spl* homozygous plants. Additionally, the flowers of the *spl* homozygous plants were found to have a normal number of organs, as in the wild type plants, except that the flowers of the *spl* homozygous plants included white, flat anthers and lacked visible pollen grains at anthesis in stage 13-14. See D.R. Smyth, J.H. Bowman, E.M. Meyerowitz, 1990, *Plant Cell* 2, 755. The carpel of these *spl* homozygous plants also appeared morphologically normal, although being infertile when pollinated with wild type pollen grains.

Cytological studies using whole mount clearing and sectioning techniques demonstrated that meiocyte formation was affected in both anther and carpel of the *spl* homozygous plants. Studies of the *spl* homozygous plants also revealed that in *spl* mutant flowers the hypodermal cell of the anther enlarged slightly in stage 7 and differentiated into an archesporial cell, as occurs normally in wild type flowers. The archesporial cell then differentiated and sometimes divided periclinally to form the PPC layer and the PSC layer. The PPC layer occasionally divided an additional time to produce two secondary parietal cell layers that ceased dividing. However, cells closer to

the center of the anther became vacuolated, and the development of microsporocytes and tapetum was not observed. Additionally, at anthesis in stages 13-14, the anthers were composed of highly vacuolated parenchymatous cells, and in some cases, several vascular cells also were present.

In contrast, the results of the above studies differed from those of the wild type *Arabidopsis* in which the wild type was found to exhibit microsporogenesis as typically exhibited by dicotyledonous plants. Specifically, in immature flowers at stage 7, a single hypodermal cell at each corner of the anther locules expanded radially and differentiated into an archesporial cell. The archesporial cell underwent a periclinal division, resulting in an inner primary sporogenous cell (PSC) layer and an outer primary parietal cell (PPC) layer. The PPC layer subsequently divided periclinally and anticlinally to form two secondary parietal cell (SPC) layers, while the inner SPC layer differentiated into the tapetum. The outer SPC layer then divided periclinally an additional time to form two more layers called the endothecium, which lies outside, and the middle layer, which lies inside. None of these layers that are descended from the PPC or primary parietal cell layer have any direct role in spore formation, although they are important for maturation of the pollen grains. The spores were formed from the cells of the PSC layer (primary sporogenous layer) which differentiated directly into microsporocytes (male meiocytes), also referred to as pollen mother cells (PMCs) in late stage 8 flowers. During stage 9, the

PMCs separated from one another by the deposition of callose on the cell wall, and subsequently underwent meiosis. See Bowman, J., 1994, *Arabidopsis, An Atlas of Morphology and Development*. At the same time, the tapetum became visible and appeared binucleate due to endomitosis.

It was concluded from the above studies with the *spl* mutant in comparison to the wild type plants that microsporogenesis in *spl* mutant plants is blocked during the transition from the PSC layer to microsporocytes, resulting in a phenotype lacking any microsporocytes.

In the *spl* mutants studied in accordance with the present invention it also was found that the ovule primordia formed normally, and the top hypodermal cell increased slightly in size. The archesporial cell was formed as in the wild type plant, but was unable to elongate longitudinally to develop into megasporocyte or female meiocyte. Therefore, the *spl* mutant failed to form megasporocyte, and as a result, the nucellus became arrested. However, both inner and outer integuments differentiated normally as in wild flower type flowers. The endothelium also differentiated from the inner cell layer of the inner integument. Shortly after the integument developed in stage 13 flowers, the top epidermal cell of the arrested nucellus elongated and started to divide transversely and mitotically, and thereafter the two neighboring epidermal cells also divided transversely. As a result, the nucellus grew towards the micropyle to produce a three layered finger-like structure on a longitudinal section of the

ovule in and after stage 14 flowers.

The *spl* mutation prevented the transition from the archesporial cell to megasporocyte during megasporogenesis, which was evidenced in part by the absence of callose deposition on carpel at different flower stages, as observed during wholemount staining with aniline blue. However, this did not affect the development of sporophytic tissues such as integument, thus indicating that the *spl* mutation specifically blocked the transition from the archesporial cell into megasporocyte in the plant.

The *SPL* gene product thus appears to play a pivotal role in the formation of microsporocytes in the male plant and megasporocytes in the female plants. Genetic studies, including Southern blot analysis using the 5' Ds sequence as probe, showed that the *spl* sterile phenotype was caused by a single Ds insertion. Additional reversion experiments confirmed that the *spl* mutant gene is tagged by the Ds element. Excision of this Ds element by the Ac transposase gene restored sporocyte formation and normal fertility. In these experiments, ten independent revertant plants, which were fully fertile, were isolated. In each instance, it was determined that the Ds element within the SPL gene had undergone precise excision, restoring the wild-type sequence and function.

Genomic sequences flanking the Ds element were detected by using the thermal asymmetric interlaced-PCR (TAIL PCR) technique, as described by Liu, *et al.*, *The Plant J.*, 8:457 (1995). As shown in Figures 1A and 1B [SEQ ID NOS:2 and 3], fragments immediately flanking

each of the 3' and 5' ends of the Ds element were sequenced and found to contain, as expected, the 3' and 5' portions of the Ds sequence. The above PCR fragments were used as a probe to screen a cDNA library from *Arabidopsis thaliana* Landsberg *erecta* flower. A cDNA clone of the *SPL* gene was isolated and sequenced. As shown in Figure 2 [SEQ ID NO:1], the full-length cDNA clone was found to be 1302bp in length and to encode a 314 amino acid polypeptide having a molecular weight of 34 kDa, as shown in Figure 3 [SEQ ID NO:4].

Additionally, searches of databases of protein sequences revealed that the SPL protein, as shown in Figure 3 [SEQ ID NO:4], was not homologous to any known proteins, thus confirming the novelty of the SPL protein. Partial homologies to amino acid regions of known proteins are by three short regions of the SPL protein. Specifically, one 33 amino acid domain from positions 149 to 181 of the *SPL* protein was found to be homologous to an amino acid region of *Sacchromyces cerevisiae* SWE1, a mitosis inhibitor, with 45% identity. Another 15 amino acid region from positions 119 to 133 of the *SPL* protein was found to be homologous, with 73% identity, to an amino acid region of 3-hydroxyisobutyrate dehydrogenase precursor from rat. However, both of the above amino acid regions are from unrelated proteins and have an unknown function.

Sub A57 In addition, there is a predicted helix region in SPL protein from amino acids 64 to 85 that has limited homology with the first helix region of the protein motif called the MADS domain that binds DNA. The MADS domain is a highly conserved region of about 57 amino

~~acids found in a family of transcription factors called MADS box factors (See, *e.g.*, Kramer et al., *Genetics* 149:765-783 (1998)). SPL does not have the entire MADS domain, but it shows good conservation to the first 18 amino acids of this domain. A comparison of amino acids 64 to 80 of SPL with the first amino acids of the MADS domain from known regulatory proteins of this class from a variety of species is shown in Figure 4 (SEQ ID NOS:5-14).~~

Sub A67 ~~As shown in Figure 4, the MADS box transcription factors listed are the AP3, AG, AGL5 and AGL11 proteins of Arabidopsis; DEFA and GLO proteins of Antirrhinum (snapdragon); BOAP1 from Brassica oleracea; FBP11 from petunia; MCM1, RLM1, SMP1 proteins from budding yeast; and SRF and MEF2D human proteins.~~

The nuclear localization of SPL and its partial homology with the previously described MADS domain proteins suggest that SPL may represent a new class of transcription regulatory protein.

Northern blot analysis of polyA$^+$ RNAs from flowers, roots, leaves, stems and silique of *Arabidopsis* using the above-described cDNA clone as a probe revealed a 1.3kb band only in RNA extracted from the flower, thus suggesting that the *SPL* gene is expressed differently in different plant tissues. *In situ* hybridization using as the probe labeled antisense RNA, synthesized from the *SPL* cDNA clone, also demonstrated that the *SPL* gene is expressed in sporogenous cells in flowers, which is consistent with the biological function of the gene.

As shown in Figures 1A, 1B [SEQ ID NOS:2 and 3]

and 2 [SEQ ID NO:1], a comparison of genomic sequences with a cDNA sequence from *Arabidopsis* revealed that the Ds element is inserted between bases 411 and 412 of the *SPL* gene. This insertion of the Ds element caused a 4bp duplication of the host sequence at the insertion site. Sequences obtained from more than 10 independent reversion lines revealed a perfect excision, and no footprints, thus indicating the importance of the region comprising the amino acids immediately flanking the insertion site to the function of the *SPL* gene. this conclusion was based upon the observation that all revertants of the *spl* mutation were precise excisions of the Ds element. When a Ds element is inserted into a gene, there typically are revertants in which there are small deletions, substitutions or insertions of one or two amino acids (Wessler, S.R., *Science* 242(4877):399-405 (October 21, 1988)). That no such revertants were recovered from the *spl* mutation is evidence that even small changes in the amino acid sequence at the site of insertion are deleterious to the function of the SPL protein.

Southern hybridization analysis showed that the *SPL* gene is a single gene.

In accordance with the present invention, the *Sporocyteless* (*SPL*) gene from *Arabidopsis thaliana* thus appears to play an important, if not essential, role in the transition from archesporial cells to meiocytes in both male and female organs of plants. As stated above, sporogenesis is a key step in the reproduction of a plant, and thus the ability of a plant to control sporogenesis also affects the plant's ability to yield

seeds. The genetic studies of the *spl* mutation of *Arabidopsis* described herein show that the *SPL* gene encodes a protein that is important, if not essential, for meiocyte formation. Using transposon tagging, the *SPL* gene was isolated and characterized. Additional Southern analysis under moderate stringency levels should reveal *SPL* homologues in other plant species, such as maize and rice, having the same or similar function as the *SPL* gene.

As stated above, the isolated DNA provided by this invention may be used as a probe to isolate in other plant species DNA sequences that are homologous to the *SPL* gene and encode regulatory proteins which are involved in meiocyte formation in the same or similar way as is protein encoded by the *SPL* gene. As stated above, the terms "homology" and "homologous" in the present invention mean an overall sequence identity of at least 50%. The identification and isolation of *SPL*-type genes (i.e., homologues of the *SPL* gene) of other plant species may be carried out according to standard methods and procedures known to those skilled in the art. See, e.g., Sambrook, *et al. Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

By using these and other similar techniques, those skilled in the art can readily isolate not only the *SPL* gene from different cells and tissues of *Arabidopsis*, but also homologues of the *SPL* gene from other plant species. For example, *SPL* or *SPL*-type genes in other plant species may be identified and isolated by preparing a genomic and/or cDNA library of the plant

species, followed by probing either or both of the libraries with all or a portion of either of the sequences shown in Figures 1A [SEQ ID NO:2] and 2 [SEQ ID NO:1], or their homologues, identifying the hybridized sequences, and isolating the hybridized DNA to obtain the *SPL* or *SPL*-type gene. Once identified, these *SPL* or *SPL*-type genes from other plant species may be restriction mapped, sequenced and cloned.

The isolated *SPL* gene, or a homologue thereof, also may be altered and thereafter introduced into *Arabidopsis* or another plant species to regulate and control meiocyte formation to produce seedless fruits and/or pollenless plants. For example, an engineered *SPL* gene may be incorporated into a plant line, which has been bred for other traits, to produce seedless fruits.

Meiocyte formation also can be blocked by decreasing the expression levels of SPL protein by using antisense constructs or co-suppression of the *SPL* gene. Alternatively, by placing the sense or antisense *SPL* gene under the control of different inducible promoters, meiocyte formation also can be controlled, subject to specific environmental conditions or applied chemicals.

"Cosuppression" refers to the over-expression of an endogenous or introduced exogenous gene (transgene), wherein the extra copies of the gene cause coordinate silencing of both the endogenous gene and transgene, thus reducing or eliminating expression of a certain trait. See, e.g., U.S. Patent Nos. 5,034,323 and 5,283,184. The transgene can be introduced in a sense

or antisense orientation and does not require a full-length sequence or absolute homology to the endogenous sequence intended to be repressed.

Furthermore, a dominant-negative mutant of the SPL protein can be constructed by using a truncated version of the *SPL* gene that is able to interact with its partners, but is unable to fulfill its biological activity. See Wilkinson, J.Q., et al., *Nature Biotechnology* 15(5):444-447 (1997). If this truncated gene is introduced into a plant under the control of a strong promoter, the transgenic plant should reduce or lose its ability to form seeds. Therefore, a truncated dominant-negative *SPL* gene could act as a substitute for the antisense *SPL* gene. The dominant-negative *SPL* gene approach also has advantages over the antisense construct when engineering seedless or pollenless plants, including that the antisense strategy depends on initially cloning part or all of the *SPL* gene from each plant species, followed by expressing the gene in an inverted orientation. Antisense suppression also is dependent on the expression of the complementary nucleotide sequences, which vary from one species to another. In contrast, the dominant-negative strategy is dependent only on the functional conservation of the protein and its target sites, which is a much less stringent requirement overall than is nucleotide sequence conservation. There are several examples of regulatory proteins that can perform a similar function when expressed in widely divergent species of plants, as discussed in Lloyd, A.M. *et al.*, (1992), *Science* 258: 1773-1775; Irish, V.F. and Yamamoto, Y.T., (1995),

Plant Cell 7: 1635-1644. This type of functional conservation suggests that the dominant-negative version of the *Arabidopsis SPL* gene also can work similarly in other plant species.

The following examples describe specific aspects of the invention to illustrate the invention and describe methods for isolating and identifying the *SPL* gene. The examples should not be construed as limiting the invention in any way.

All citations in this application, including those to materials and methods, are hereby incorporated by reference.

EXAMPLE 1

TRANSPOSON TAGGING

Plants were grown at 22°C under 16hr light/8hr dark cycle in green houses at the Institute of Molecular Agrobiology, 1 Research Link, Singapore. Starter lines containing Ds or Ac segments were crossed and screened for transposants of F2 seeds, according to Sundaresan, V., *et al.*, 1995, *Genes & Development*, 9:1797-1810. The *spl* mutant gene was identified from among a collection of transposants by its male and female sterile phenotypes. Genetic analysis was carried out using techniques recognized in the art. The *spl* mutant gene was shown to be recessive and caused by a single Ds insertion. The phenotype of the *spl* mutant gene was characterized by standard cytological methods, as discussed, for example, in O'Brien, T.P. and McCully, M.E., 1981, *The Study of Plant Structure: Principles and Selected Methods*, Termarcarphi, Melbourne; and by wholemount clear methods, as discussed in Herr, J.J.M., 1982, *Stain Technol. 57*: 161-169.

EXAMPLE 2

DNA ANALYSIS

DNA analysis procedures were performed primarily as described in Sambrook, J., *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor Laboratory, New York.

For Southern blot analysis, 100-200ng *Arabidopsis* DNA was extracted from flower buds and digested with EcoRI, Hind III, or Xba I and electrophoresed on a 1%

agarose gel prior to transfer to a nylon membrane. The Ds probe, an EcoRI fragment from the 5' end of the gene trap construct, *DsG* (see V. Sundaresan et al., *Genes & Development* 9:1797-1810 (1995)), was prepared by digesting the plasmid pWS31, which contained parts of Ds elements, with EcoRI and separating the resulting fragments by gel electrophoresis. A 1.8kb EcoRI fragment of a 5' Ds element was cut from the gel and labeled with ^{32}P-dCTP, using the Rediprime kit from Amersham. The labeled fragment was used to probe Southern blots under standard DNA hybridization conditions.

To isolate the DNA immediately flanking the Ds element, about 10ng DNA from flower buds was used for TAIL PCR (Liu, *et al.*, 1995, *The Plant J.* 8, 457). The amplified fragments were isolated by gel electrophoresis and sequenced. The PCR fragments were labeled with ^{32}P-dCTP and used to screen a flower cDNA library. Phages in the library that hybridized to the PCR fragments were purified, and plasmid DNA was excised *in vitro* according to a standard protocol. The size of the insert was determined by digesting the plasmid with the restriction enzymes EcoRI and KpnI, both available from Stratagene.

EXAMPLE 3

RNA ANALYSIS

Northern blot analysis of polyA+ RNA from various *Arabidopsis* tissues was performed using a 1kb Hind III fragment of the cDNA clone of Figure 2 [SEQ ID NO:1] as a probe. RNA was extracted from different tissues

using standard methods. 10µg polyA+ RNA from each sample was electrophoresed on 1% agarose gel and transferred to a nylon membrane. The membrane was then hybridized with a ^{32}P-dCTP labeled probe.

EXAMPLE 4

SEQUENCING OF THE *SPL* GENE

The SPL cDNA clone of Figure 2 [SEQ ID NO:1] was sequenced using the dideoxy method with fluorescent labeled terminators. T3 and T7 oligonucleotide primers, which hybridized to the plasmid vector containing the *SPL* cDNA, were used to generate initial sequences from the ends of the clone. Additional primers within the *SPL* gene, based on the above sequences, were then designed and used to sequence the central region of the *SPL* gene. Approximately 600-700 bp of the clone could be read from each primer.

EXAMPLE 5

IN SITU LOCALIZATION OF THE *SPL* mRNA

Flower buds were fixed with FAA for 20 hours at 4°C, dehydrated with ethanol and made transparent with xylene. The tissues were embedded in paraplast and 7-10µm thick sections were made. The sections were then deparaffinized with xylene and processed for *in situ* hybridization. To obtain sense RNA probe, the plasmid containing the *SPL* cDNA was linearized with Kpn I and transcribed with T3 RNA polymerase in the presence of DIG-UTP. For antisense RNA probe, the plasmid was cut with BamHI and transcribed in the presence of DIG-UTP

with T7 RNA polymerase. The lengths of the probes were reduced by alkaline treatment to a fragment having a length of about 150 bp. Hybridization was performed according to a standard protocol. See Jackson, D., 1991, In situ Hybridization in Plants, in *Plant Pathology: A Practical Approach*, Oxford University Press.

EXAMPLE 6

DETERMINATION THAT SPL PROTEIN IS A NUCLEAR PROTEIN

It has been determined that the SPL protein is a nuclear protein. A translational fusion of the SPL protein to the GUS reporter gene (Jefferson, R.A., *Nature* 342(6251):837-8 (Dec., 1989) was utilized for this purpose. The method used for determining the nuclear localization has been previously described for other proteins (*e.g.*, Pepper et al., *Cell* 78(1):109-116 (1994)).

Sub A7

Two primers, SPL-Xba-S:5'CTAGTCTAGTCTAGAAGATCATCA3' [SEQ ID NO.16] and SPL-BamH1-T:5'CGGATCCAAGCTTCAAGGACAAATCAATGGT3' [SEQ ID NO:17], which introduced restriction enzyme sites immediately upstream of the *SPL* start codon and the *SPL* stop codon, respectively, were used to amplify the complete *SPL* coding sequence from the cDNA. This amplified fragment was cloned in front of the GUS gene in the pBI221 vector (Clontech), giving rise to clone pBI221-SPL, which encodes a SPL-GUS fusion. The gene fusion in pBI221-SPL is driven by the 35S promoter and will result in the synthesis in plant cells of a fusion protein consisting of the complete SPL protein at the N

terminus and the GUS protein at the C terminus.

The pBI221-SPL plasmid DNA was introduced into onion epidermal cells using the BioRad PDS-1000/He particle bombardment system. The samples were kept overnight at room temperature and stained with X-Gluc, a histochemical stain for GUS activity (Jefferson, R.A., *Nature* 14:342(6251):837-8 (Dec., 1989)). The SPL-GUS fusion protein was found to be localized exclusively in the nucleus, whereas in the same experiment a control GUS protein with no fusion was localized to the cytoplasm. This experiment demonstrates that SPL is a nuclear protein, which is consistent with its proposed function as a regulatory protein required for sporocyte development.

EXAMPLE 7

PROMOTER OF THE *SPL* GENE

A fragment of DNA from 2690 nucleotides upstream of the start codon of the *SPL* gene was fused to a promoterless GUS gene in a binary T-DNA vector designated pZIP111 (Hajdukiewicz, P., et al., *Plant Mol. Biol.* 25:989-994 (1994) for plant transformation. The *SPL* promoter-GUS co-construct was introduced into Landsberg plants by vacuum infiltration and transformed plants were selected by standard methods (*e.g.*, Bechtold, N., and Pelletier, G., *Methods Mol. biol.* 82:259-266 (1998)). A histochemical staining procedure was used to monitor expression of the GUS reporter gene (Jefferson, R.A., *Nature* 342(6251):837-8 (Dec., 1989)). The transgenic plants showed expression of the GUS reported gene in the megasporocytes and

microsporocytes. The pattern of GUS expression observed was similar to the expression pattern of the *SPL* gene, as determined by in situ localization of *SPL* RNA (see Example 5, above). This experiment showed that the 2690 base pairs of DNA upstream of the *SPL* start codon contain the *SPL* promoter region, and that this sequence of DNA was sufficient to confer the specificity of expression of the *SPL* gene (i.e., expression in sporocytes) to a heterologous transgene such as the GUS gene.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

What is claimed is:

1. An isolated nucleic acid or its complement comprising nucleic acid encoding a protein according to SEQ ID NO:4.

2. An isolated nucleic acid or its complement according to claim 1, wherein said nucleic acid comprises a nucleic acid as set forth in SEQ ID NO:1.

3. An isolated nucleic acid or its complement according to claim 2, wherein said nucleic acid comprises a nucleic acid as set forth in nucleotides 81-1024 of SEQ ID NO:1.

4. An isolated nucleic acid or its complement encoding a protein which participates in meiocyte formation in a plant, wherein said nucleic acid comprises naturally occurring DNA, or DNA degenerate to said naturally occurring DNA, from a plant that hybridizes to the DNA of (a) SEQ ID NO:2 or SEQ ID NO:3, or portions thereof, or (b)SEQ ID NO:1, or portions thereof, under moderately stringent conditions, wherein the naturally occurring DNA has at least about 70% identity to the DNA of (a) or (b), and wherein said naturally occurring DNA encodes said protein.

5. An isolated nucleic acid or its complement according to claim 4 having at least about 70% identity to (a) nucleotides 81 - 1024 of SEQ ID NO:1, or a portion thereof, or (b) variations of (a) which encode the same amino acid sequence as encoded by (a), but

employ different codons for some of the amino acids.

6. An isolated nucleic acid encoding a protein involved in meiocyte formation in a plant, wherein said protein comprises:

(a) the same amino acid sequence as set forth in SEQ ID NO:4, or

(b) an amino acid sequence having at least 80% homology to the amino acid sequence as set forth in SEQ ID NO:4 and which is involved in meiocyte formation in a plant.

7. An isolated nucleic acid according to any one of claims 1 to 6, wherein said nucleic acid is mutated to block, reduce, or increase formation of meiocytes in a plant.

8. An isolated nucleic acid according to claim 7, wherein said nucleic acid is mutated by insertion of one or more genetic elements.

9. An isolated nucleic acid according to claim 8, wherein said genetic elements comprise a Ds sequence.

10. A protein required for the formation of meiocytes in a plant, wherein said protein comprises:

(a) the same amino acid sequence as set forth in SEQ ID NO:4, or

(b) an amino acid sequence having at least 80% homology to the amino acid sequence as set forth in SEQ ID NO:4 and which is involved in the formation of meiocytes in a plant.

11. An antibody which recognizes and binds to a protein according to claim 10.

12. A fusion protein comprising any one of the amino acid sequences according to claim 10.

13. A plant transformed with an isolated nucleic acid sequence or its complement comprising nucleic acid encoding a protein according to SEQ ID NO: 4.

14. A plant transformed with an isolated nucleic acid sequence or its complement according to claim 13, wherein said nucleic acid sequence comprises a nucleic acid as set forth in SEQ ID NO:1.

15. A plant transformed with an isolated nucleic acid sequence or its complement according to claim 14, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in nucleotides 81-1024 of SEQ ID NO:1.

16. A plant according to claim 13, 14 or 15 wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes in said plant.

17. A plant seed transformed with an isolated nucleic acid sequence or its complement comprising nucleic acid encoding a protein according to SEQ ID NO: 4.

18. A plant seed transformed with an isolated nucleic acid sequence or its complement according to claim 17, wherein said nucleic acid sequence comprises

a nucleic acid as set forth in SEQ ID NO:1.

19. A plant seed transformed with an isolated nucleic acid sequence or its complement according to claim 18, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in nucleotides 81-1024 of SEQ ID NO:1.

20. A plant seed according to claim 17, 18 or 19 wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes in a plant.

21. A plant cell transformed with an isolated nucleic acid sequence or its complement comprising nucleic acid encoding a protein according to SEQ ID NO: 4.

22. A plant cell transformed with an isolated nucleic acid sequence or its complement according to claim 21, wherein said nucleic acid sequence comprises a nucleic acid as set forth in SEQ ID NO:1.

23. A plant cell transformed with an isolated nucleic acid sequence or its complement according to claim 22, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in nucleotides 81-1024 of SEQ ID NO:1.

24. A plant cell according to claim 21, 22 or 23 wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes in a plant.

25.A method of producing a transgenic plant which is capable of bearing substantially seedless fruits or substantially pollenless flowers, comprising the step of transforming a plant with a nucleic acid or its complement comprising nucleic acid encoding a protein according to SEQ ID NO: 4.

26. A method of producing a transgenic plant which is capable of bearing substantially seedless fruits or substantially pollenless flowers, comprising the step of transforming a plant with a nucleic acid sequence or its complement according to claim 25, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in SEQ ID NO:1.

27. A method of producing a transgenic plant which is capable of bearing substantially seedless fruits or substantially pollenless flowers, comprising the step of transforming a plant with a nucleic acid sequence or its complement according to claim 26, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in nucleotides 81-1024 of SEQ ID NO:1.

28. A method according to claim 25, 26 or 27, wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes in said plant, thereby rendering said plant capable of bearing said seedless fruits or pollenless flowers.

29. A method according to claim 28, wherein said nucleic acid is a nucleic acid according to claim 27.

30. A method according to claim 28, wherein said

nucleic acid is mutated by insertion of one or more genetic elements.

31. A method according to claim 30, wherein said genetic elements comprise a Ds sequence.

32. A method of producing substantially seedless fruits or substantially pollenless flowers in a plant, comprising the step of expressing in said plant an isolated nucleic acid sequence or its complement comprising a nucleic acid sequence encoding a protein according to SEQ ID NO:4, wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes and thereby produce said seedless fruits or pollenless flowers in said plant.

33. A method of producing substantially seedless fruits or substantially pollenless flowers in a plant, comprising the step of expressing in said plant an isolated nucleic acid sequence or its complement according to claim 32, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth in SEQ ID NO:1,
wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes and thereby produce said seedless fruits or pollenless flowers in said plant.

34. A method of producing substantially seedless fruits or substantially pollenless flowers in a plant, comprising the step of expressing in said plant an isolated nucleic acid sequence or its complement according to claim 33, wherein said nucleic acid sequence comprises a nucleic acid sequence as set forth

in nucleotides 81-1024 of SEQ ID NO:1, wherein said nucleic acid is mutated to block, reduce or increase the formation of meiocytes and thereby produce said seedless fruits or pollenless flowers in said plant.

35. A method according to claim 32, 33 or 34, wherein said nucleic acid is mutated by insertion of one or more genetic elements.

36. A method according to claim 35, wherein said genetic elements comprise a Ds sequence.

37. An isolated nucleic acid or its complement useful as a hybridization probe, wherein said nucleic acid comprises a nucleic acid having a sequence of nucleotides as set forth in SEQ ID NO:2 or SEQ ID NO:1, or a portion thereof.

38. A method of producing a plant capable of bearing substantially seedless fruits or substantially pollenless flowers, comprising the step of mutating endogenous DNA of said plant responsible for the formation of meiocytes, wherein said meiocyte formation is blocked, reduced or increased and said plant becomes capable of producing said seedless fruits or pollenless flowers.

39. A method according to claim 38, wherein said endogenous DNA is mutated by direct mutagenesis.

40. An isolated nucleic acid or its complement comprising nucleic acid coding for a mutant SPL polypeptide which blocks, reduces or increases the

formation of meiocytes in a plant.

41. An isolated DNA comprising DNA having at least 8 consecutive nucleotides of bases 81 - 1024 of SEQ ID NO:1, or a complement thereof.

42. The isolated DNA of claim 41, wherein said DNA has at least 15 consecutive nucleotides of bases 81 - 1024 of SEQ ID NO:1.

43. An isolated DNA wherein said isolated DNA consists of 8 or more consecutive nucleotides of a sequence of nucleotides 81 - 1024 of SEQ ID NO:1, or a complement thereof.

44. The isolated DNA of claim 43, wherein said DNA consists of 15 or more consecutive nucleotides of a sequence of nucleotides 81 - 1024 of SEQ ID NO:1.

Sub A87

~~45. An isolated nucleic acid sequence comprising a nucleic acid sequence as set forth in nucleotides -2690 to -1 of SEQ ID NO. 15 or a nucleotide sequence which hybridizes to said sequence and promotes expression of a coding sequence operably linked to said nucleotide sequence.~~

~~46. An isolated nucleotide sequence or functional fragments thereof capable of regulating expression of an operably linked gene, said sequence comprising a nucleotide sequence located within nucleotide positions -2690 to -1 of the nucleotide sequence set forth in SEQ ID NO: 15 or a nucleotide sequence which hybridizes to said sequence and promotes expression of an operably linked gene.~~

~~47. An isolated DNA fragment for directing the expression of a foreign or endogenous gene in a cell, said fragment comprising a sequence as set forth in nucleotides -2690 to -1 of SEQ ID NO: 15 operably linked to an ATG start codon of a foreign or endogenous gene.~~

48. An isolated nucleotide sequence as set forth in claim 45 or 46, operably linked to a foreign or endogenous functional gene.

Sub A97 ~~49. A method for regulating the expression of a gene which comprises providing a gene of interest operably linked to an *SPL gene* promoter, transferring said operably linked gene to a cell and expressing said gene under gene expression conditions, wherein said *SPL* gene promoter comprises a nucleotide sequence located within nucleotide positions -2690 to -1 of the nucleotide sequence set forth in SEQ ID NO:15 or a nucleotide sequence which hybridizes to said sequence and promotes expression of an operably linked gene.~~

50. The method of claim 49, wherein said cell is a reproductive cell of a plant.

51. The method of claim 50, wherein said reproductive cell is a sporocyte.

52. The method of claim 50, wherein said gene encodes a ribonuclease, a transposase or a recombinase.

53. A plant comprising cells transformed with a foreign gene operably linked to and under the control of a nucleotide sequence as set forth in claim 45 or 46.

5' of the *SPL* gene

gtagcatcga tctctaacaa cgctacccgt ttacccgtac cggtagaccc gggtgttgt 59

3' of Ds element

gcta<<<**cagggat gaaaacggtc ggtaacggtc ggtaaaatac------------------**

-------------------Ds element------------------------------------

tacgggattt ttcccatcct actttcatcc cgg>>>gctacaa ggcttcccaa

5' of Ds element

gctcatcggg agcaacagga tctattgtgg tggagtcggg tcgggtcagg ttatgatcga
cccggttatt tctccatggg gttttgttga gacctcctcc actactcatg agctctcttc a

FIG. 1A.

cagggat gaaaacggtc ggtaacggtc ggtaaaatac tacgggattt ttcccatcct
actttcatcc cgg

FIG. 1B.

CAGACTTAAAGCTTTCGTCTTTACCTCTTGCCTTCTCTCTCTCTATCTAAAAAGAGTTCCGAGA 64
AGAAGATCATCATCAATGGCGACTTCTCTCTTCTTCATGTCAACAGATCAAAACTCCGTCGGAA 128
ACCCAAACGATCTTCTGAGAAACACCCGTCTTGTCGTCAATAGCTCCGGCGAGATCCGGACAGA 192
GACACTGAAGAGTCGTGGTCGGAAACCAGGATCGAAGACAGGTCAGCAAAAACAGAAGAAACCA 256
ACGTTGAGAGGAATGGGTGTAGCAAAGCTCGAGCGTCAGAGAATCGAAGAAGAAAAGAAGCAAC 320
TCGCCGCCGCCACAGTCGGAGACACGTCATCAGTAGCATCGATCTCTAACAACGCTACCCGTTT 384
ACCCGTACCGGTAGACCCGGGTGTTGTGCTACAAGGCTTCCCAAGCTCACTCGGGAGCAACAGG 448
ATCTATTGTGGTGGAGTCGGGTCGGGTCAGGTTATGATCGACCCGGTTATTTCTCCATGGGGTT 512
TTGTTGAGACCTCCTCCACTACTCATGAGCTCTCTTCAATCTCAAATCCTCAAATGTTTAACGC 576
TTCTTCCAATAATCGCTGTGACACTTGCTTCAAGAAGAAACGTTTGGATGGTGATCAGAATAAT 640
GTAGTTCGATCCAACGGTGGTGGATTTTCGAAATACACAATGATTCCTCCTCCGATGAACGGCT 704
ACGATCAGTATCTTCTTCAATCAGATCATCATCAGAGGAGCCAAGGTTTCCTTTATGATCATAG 768
AATCGCTAGAGCAGCTTCAGTTTCTGCTTCTAGTACTACTATTAATCCTTATTTCAACGAGGCA 832
ACAAATCATACGGGACCAATGGAGGAATTTGGGAGCTACATGGAAGGAAACCCTAGAAATGGAT 896
CAGGAGGTGTGAAGGAGTACGAGTTTTTTCCGGGGAAATATGGTGAAAGAGTTTCAGTGGTGGC 960
TACAACGTCGTCACTCGTAGGTGATTGCAGTCCTAATACCATTGATTTGTCCTTGAAGCTTTAA 1024
ATGTTTTATCTTTCTATATTGATTTAAACAAAATCGTCTCTTTAAAGAAAAAACATTTTAAGTA 1088
GATGAAAGTAAGAAACAGAAGAAAAAAAAGAGAGAGCCTTTTTTGGTGTATGCATCTGAGAGCT 1152
GAGTCGAAAGAAAGATTCAGCTTTTGGATTACCCTTTTGGTTGTTTATTATGAGATTCTAACCT 1216
AAACACTCAGACATATATGTTCTGTTCTCTTCCTTAATTGTTGTCATGAAACTTCTCAAAAAAA 1280
AAAAAAAAAAAAAAAAAAAAAA 1302

FIG.2

Met Ala Thr Ser Leu Phe Phe Met Ser Thr Asp Gln Asn Ser Val Gly Asn Pro Asn Asp
1 5 10 15 20
Leu Leu Lrg Asn Thr Arg Leu Val Val Asn Ser Ser Gly Glu Ile Arg Thr Glu Thr Leu
25 30 35 40
Lys Ser Arg Gly Arg Lys Pro Gly Ser Lys Thr Gly Gln Gln Lys Gln Lys Lys Pro Thr
45 50 55 60
Leu Arg Gly Met Gly Val Ala Lys Leu Glu Arg Gln Arg Ile Glu Glu Glu Lys Lys Gln
65 70 75 80
Leu Ala Ala Ala Thr Val Gly Asp Thr Ser Ser Val Ala Ser Ile Ser Asn Asn Ala Thr
85 90 95 100
Arg Leu Pro Val Pro Val Asp Pro Gly Val Val Leu Gln Gly Phe Pro Ser Ser Leu Gly
105 110 115 120
Ser Asn Arg Ile Tyr Cys Gly Gly Val Gly Ser Gly Gln Val Met Ile Asp Pro Val Ile
125 130 135 140
Ser Pro Trp Gly Phe Val Glu Thr Ser Ser Thr Thr His Glu Leu Ser Ser Ile Ser Asn
145 150 155 160
Pro Gln Met Phe Asn Ala Ser Ser Asn Asn Arg Cys Asp Thr Cys Phe Lys Lys Lys Arg
165 170 175 180
Leu Asp Gly Asp Gln Asn Asn Val Val Arg Ser Asn Gly Gly Gly Phe Ser Lys Tyr Thr
185 190 195 200
Met Ile Pro Pro Pro Met Asn Gly Tyr Asp Gln Tyr Leu Leu Gln Ser Asp His His Gln
205 210 215 220
Arg Ser Gln Gly Phe Leu Tyr Asp His Arg Ile Ala Arg Ala Ala Ser Val Ser Ala Ser
225 230 235 240
Ser Thr Thr Ile Asn Pro Tyr Phe Asn Glu Ala Thr Asn His Thr Gly Pro Met Glu Glu
245 250 255 260
Phe Gly Ser Tyr Met Glu Gly Asn Pro Arg Asn Gly Ser Gly Gly Val Lys Glu Tyr Glu
265 270 275 280
Phe Phe Pro Gly Lys Tyr Gly Glu Arg Val Ser Val Val Ala Thr Thr Ser Ser Leu Val
285 290 295 300
Gly Asp Cys Ser Pro Asn Thr Ile Asp Leu Ser Leu Lys Leu
305 310

FIG. 3

```
AP3          1  MARGKIQIKRIENQTNRQ
DEFA         1  MARGKIQIKRIENQTNRQ
AG_          1  SGRGKIEIKRIENTTNRQ
MCM1         1  KERRKIEIKFIENKTRRH
SRF          1  RGRVKIKMEFIDNKLRRY
GLO          1  MGRGKIEIKRIENSSNRQ
RLM1-yeast   1  MGRRKIEIQRISDDRNRA
SMP1-yeast   1  MGRRKIEIEPIKDDRNRT
MEF2D        1  MGRKKIQIQRITDERNRQ
AGL5         1  MGRGKIEIKRIENANSRQ
FBP11        1  MGRGKIEIKRIENNTNRQ
BOAP1        1  MGRGRVQLKRIENKINRQ
AGL11        1  MGRGKIEIKRIENSTNRQ
SPL          1  MGVAKLERQRIEEE-KKQ
```

Figure 4

FIGURE 5

-2690

cggatcccaagaatctttctatgcctgcctaaacccagcaatataaatcaaaccttcacacgct
tcggttcttctttacacgtgccggaaaaaaaccctagtagtagccgcccaatgaccatctaaa
gtggtccccgtgatgacacgtgtcagttggaccactatccgtaacttaacatgaaagcacatgt
ggggtccctctttcgtcctttgccctaccagttccttgtcctagcccacaatacaatctacgcg
gtatctatatcaaagtttatctagctatttccgaaatagaaagcatatacttccatttatttt
tgaacaaattaaacttggtagaaataaaatctttcgatattgatttatttcgatttagtgtaat
tctattatcatctcgcgtgtcattctaggcttatagcaacagtgtaggtatgttgcaatgttgg
gttggtcatgccgtttggatttatttccagtgattaattcagattttatttttcttcttaatta
tctacgtataacaaaatctcgctaaccgcagagtgaatttgcatgtcactcatgaatgttttga
gtataagaagtgagtaatttgttttataaatatatgaacttatgaagatacatattgaagttgt
tttgtttgggggtaaaaaaggttatttgagtgttatatgataactttactcagaaaacgtactt
agcaaaggtaattcgaagtacctttggaatcgagtaaatactgataactagaaaaaataagata
cataatggagaaataattaaatatatttgtatttctttttttgtttaacaacgtacgttttatta
ttagctagtatacatttacaacggttacgtagatcatataatagccatttaagatgtacaacat
ctcatctggttacttcatttatataaaaaaaaaacgaaatctcaacacatagtaatgtataatt
acttcagtggggcttctcttaagacttgtattgagaatatccatataaaacaaactttgtatta
agataattaaaattttctaatagtaggtattgggctgaagccaagattaacatggaggcagctt
taaaatgtttccttatatgatgcagccatcatttctactctactccgtagctccaaacccttct
cgtaattcacgtctctcatgctattctttttgctttcgtcctcctctcatgtgaagcaataact
atctctcgattttttttttcaaataccgaaagctaacttttcaaataaatgtcaaatatatta
attttcgttttgtatttagtattttatttgtcagctaagtatagtgagtttttaagcttactcg
tcgtatttatcatatattcatatacatatcacattagtcaaagtaaataaaaatttgtttttga

Figure 5 (cont)

agaaaaaaaaaatacatataactgcgagtctgcgactgtaactggacttgcttattttagttga
tatgagctgagtaaaatcacgttgtcccagaccttgctcgctacaatcggcgaatggtctaacg
tcccgacacctgtcctcgatccgcgggtactatattctttgcaatgtgatgcacgcgctgttac
tattggacagtgtttctcacctcacgactgagcctatgcgagtagcgacaatctccgatttgct
gtctccatggtagggattatcacaatctctgatttttttatcaggaacaagtaaataaatagc
tttgagttttgtttttttctacattcttcgcccaaaagatgtaagaaaataaaggatttgaa
accttgttctgttgttactcctttaaattcttaaaaactataaatcattatatctttgatctgt
ttcacaaactaatcatattcgttgcaaagtgagaattcgtcccactttactctttacaccgata
ctagtattatagatgtacagcatagtattccatatctagttatttagtcaaactctatatatt
aagaggtaggttaattaattaaggagtaattgaagattatagaaagaataaaaaataccattta
atggacagaaccaaagataactaactatcatactataatgttgaatttcttccacgatccaatg
catggataacaacatcaatcaaatcatacattcatgctatataacatagttttcagttacaaac
tctctttttatttatttcagttgttccttttcatgaccatattaacatcaaataatgcatttt
tttcaacgtctcttgacttacacccactaatattgacaattgaacatctatacgactatacac
acataagttaaaaatgcatgcaagtgctaagggaatttataacatctaaggttaataagactaa
gaaagtataaaataagaatacgtattatgaatttatgatatactttactaatctttttgaaaaa
tactttaatttaatctactatagggggtaaaaagtaaaaaagaaataaagatacgtttatccgc
atatagtacctggaaataacagaaaataaaaacacaggtaagtactttgcctgagctagtatat
gaacactaaagagatacacacacacaaaaagagagcagaaacaaaacacacacacttaaagctt
tcgtctttacctc

+1
ttcccttctctctctctatctaaaaagagttccgagaagaagatcatcatcaatggcgacttct
ctcttcttcatgtcaacagatcaaaactccgtcggaaacccaaacgatcttctgagaaacaccc
gtcttgtcgtcaacagctccggcgagatccggacagagacactgaagagtcgtggtcggaaacc
aggatcgaagacaggtcagcaaaaacagaagaaaccaacgttgagaggaatgggtgtagcaaag
ctcgagcgtcagagaatcgaagaagaaaagaagcaactcgccgccgccacagtcggagacacgt
catcagtagcatcgatctctaacaacgctacccgtttacccgtaccggtagacccgggtgttgt
gctacaaggcttcccaagctcactcgggagcaacaggatctattgtggtggagtcgggtcgggt
caggttatgatcgacccggttatttctccatggggttttgttgagacctcctccactactcatg
agctctcttcaatctcaaatcctcaaatgtttaacgcttcttccaataatcgctgtgacacttg
cttcaaggtttgtttgtttttaatcgttttcatcaacatgattgatatatatatagtttttgc
acttgaaaaagttttgattttatttatgtaaaaaactgcagaagaaacgtttggatggtgatc

Figure 5 (cont)

agaataatgtagttcgatccaacggtggtggattttcgaaatacacaatgattcctcctccgat
gaacggctacgatcagtatcttcttcaatcagatcatcatcagaggagccaaggtttcctttat
gatcatagaatcgctagagcagcttcagtttctgcttctagtactactattaatccttatttca
acgaggcaacaaatcatacggtactaagtatagtccatttattaatactcatatataggtatat
atgtatataactgttgatcttatttgatttaactggtgggtttagggaccaatggaggaatttg
ggagctacatggaaggaaaccctagaaatggatcaggaggtgtgaaggagtacgagtttttcc
ggggaaatatggtgaaagagtttcagtggtggctaaaacgtcgtcactcgtaggtgattgcagt
cctaataccattgatttgtccttgaagctttaaatgttttatctttctatattgatttaaacaa
aatcgtctctttaaagaaaaaacattttaagtagatgaaagtaagaaacagaagaaaaaaaaga
gagagcctttttttggtgtatgcatctgagagctgagtcgaaagaaagattcagcttttggatta
cccttttggttgtttattatgagattctaacctaaacactcagacatatatgttctgttctctt
ccttaattgttgtcatgaaacttctc

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 99/00023

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C07K14/415 C07K16/16 C07K19/00 A01H5/00 A01H5/10 C12N5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AARTS M G ET AL: "Transposon tagging of a male sterility gene in Arabidopsis" NATURE, (1993 JUN 24) 363 (6431) 715-7. , XP002123556 the whole document	38,39
X	ELLIOTT R C ET AL: "AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth." PLANT CELL, (1996 FEB) 8 (2) 155-68. , XP002123557 the whole document	38,39

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1999	03.12.99.
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chakravarty, A

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] EBI Accession no. AF016585, 7 December 1997 (1997-12-07) KAKAVAS S.J. ET AL.: "Streptomyces caelestis cytochrome P-450 hydroxylase homolog (nidi) gene" XP002123559 abstract ---	41,42
T	YANG W C ET AL: "The SPOROCYTELESS gene of Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear protein." GENES AND DEVELOPMENT, (1999 AUG 15) 13 (16) 2108-17. , XP002123558 the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 99/00023

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 41-47
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 41-47

Present claims 41-47 are directed to DNA sequences comprising at least 8/15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.
These claims therefore relate to an extremely large number of possible sequences. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of these. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those the whole length of the sequences IDs 1 and 15 but not for shorter oligonucleotiudes.
These claims are directed to DNA sequences comprising at least 8 or 15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
GM/AY/R33-65		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/SG 99/00023	22/03/1999	
Applicant		
INSTITUTE OF MOLECULAR AGROBIOLOGY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

      [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

      [ ] contained in the international application in written form.
      [ ] filed together with the international application in computer readable form.
      [X] furnished subsequently to this Authority in written form.
      [X] furnished subsequently to this Authority in computer readble form.
      [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
      [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.
   [X] the text has been established by this Authority to read as follows:

   CONTROL OF SPOROCYTE OR MEIOCYTE FORMATION IN PLANTS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [ ] as suggested by the applicant.
   [X] because the applicant failed to suggest a figure.
   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 99/00023

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 41-47
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 41-47

Present claims 41-47 are directed to DNA sequences comprising at least 8/15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.
These claims therefore relate to an extremely large number of possible sequences. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of these. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those the whole length of the sequences IDs 1 and 15 but not for shorter oligonucleotiudes.
These claims are directed to DNA sequences comprising at least 8 or 15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 99/00023

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/415 C07K16/16 C07K19/00 A01H5/00
A01H5/10 C12N5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AARTS M G ET AL: "Transposon tagging of a male sterility gene in Arabidopsis" NATURE, (1993 JUN 24) 363 (6431) 715-7. , XP002123556 the whole document ---	38,39
X	ELLIOTT R C ET AL: "AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth." PLANT CELL, (1996 FEB) 8 (2) 155-68. , XP002123557 the whole document ---	38,39
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1999	03.12.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] EBI Accession no. AF016585, 7 December 1997 (1997-12-07) KAKAVAS S.J. ET AL.: "Streptomyces caelestis cytochrome P-450 hydroxylase homolog (nidi) gene" XP002123559 abstract ---	41,42
T	YANG W C ET AL: "The SPOROCYTELESS gene of Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear protein." GENES AND DEVELOPMENT, (1999 AUG 15) 13 (16) 2108-17. , XP002123558 the whole document -----	

1

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
ELLA CHEONG & G.MIRANDAH
P.O.Box 0931
Raffles 911732
SINGAPORE

RECEIVED
11 DEC 1999

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (day/month/year) 03/12/1999
Applicant's or agent's file reference GM/AY/R33-65	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/SG 99/00023	International filing date (day/month/year) 22/03/1999
Applicant INSTITUTE OF MOLECULAR AGROBIOLOGY et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Véronique Baillou

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]: "Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]: "Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]: "Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or "Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]: "Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GM/AY/R33-65	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/SG 99/00023	International filing date *(day/month/year)* 22/03/1999	(Earliest) Priority Date *(day/month/year)*
Applicant INSTITUTE OF MOLECULAR AGROBIOLOGY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.
   [ ] filed together with the international application in computer readable form.
   [X] furnished subsequently to this Authority in written form.
   [X] furnished subsequently to this Authority in computer readble form.
   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [ ] the text is approved as submitted by the applicant.
   [X] the text has been established by this Authority to read as follows:

   CONTROL OF SPOROCYTE OR MEIOCYTE FORMATION IN PLANTS

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [ ] as suggested by the applicant.
   [X] because the applicant failed to suggest a figure.
   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 99/00023

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 41-47
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 41-47

Present claims 41-47 are directed to DNA sequences comprising at least 8/15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.
These claims therefore relate to an extremely large number of possible sequences. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of these. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those the whole length of the sequences IDs 1 and 15 but not for shorter oligonucleotiudes.
These claims are directed to DNA sequences comprising at least 8 or 15 consecutive nucleotides of bases 81-1024 of Seq Id 1 or to nucleic acid sequences comprising a sequence set forth in nucleotides 2690 to -1 of Seq Id 15.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C07K14/415 C07K16/16 C07K19/00 A01H5/00
A01H5/10 C12N5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AARTS M G ET AL: "Transposon tagging of a male sterility gene in Arabidopsis" NATURE, (1993 JUN 24) 363 (6431) 715-7. , XP002123556 the whole document	38,39
X	ELLIOTT R C ET AL: "AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth." PLANT CELL, (1996 FEB) 8 (2) 155-68. , XP002123557 the whole document	38,39

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1999	03. 12. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] EBI Accession no. AF016585, 7 December 1997 (1997-12-07) KAKAVAS S.J. ET AL.: "Streptomyces caelestis cytochrome P-450 hydroxylase homolog (nidi) gene" XP002123559 abstract ---	41,42
T	YANG W C ET AL: "The SPOROCYTELESS gene of Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear protein." GENES AND DEVELOPMENT, (1999 AUG 15) 13 (16) 2108-17. , XP002123558 the whole document -----	

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